Variation in Complex Semiochemical Signals Arising From Insects and Host Plants

BRIAN H. AUKEMA,[1,2,3] JAIMIE S. POWELL,[4] MURRAY K. CLAYTON,[5] AND KENNETH F. RAFFA[6]

Environ. Entomol. 39(3): 874–882 (2010); DOI: 10.1603/EN09292

ABSTRACT Chemical communication by many insect species involves complex signals of both insect and plant origin. Much attention has been focused on the behavioral activities of these components but less on their sources of variation, despite implications for evolutionary theory and pest management. We studied variation in chemical signaling at host, tree-within-host, and beetle-on-tree scales using tunneling male pine engravers [*Ips pini* (Say)] on jack, *Pinus banksiana* Lamb, red, *P. resinosa* Aiton, and white, *P. strobus* L. pines. Pine engravers are distributed transcontinentally, and stereoisomeric ratios of their principal pheromone component ipsdienol varies regionally. Linear mixed-effects models were used to examine variation in monoterpene and pheromone volatile profiles, determined by gas chromatography. Phloem from white pine had the greatest concentration of monoterpenes, although insects tunneling in white pine produced the smallest ratios of monoterpenes to pheromones (1:2) in their volatile plumes relative to jack and red pine (1:1). Beetle-to-beetle variation in plume composition was ≈2–9 times greater than the inter-tree variation within a tree species. The stereoisomeric ratio of ipsdienol was highly consistent within the pheromone component of the plume. The little variation present existed almost entirely at the level of the insects. Within the pheromone component of the plume in a given host species, there was up to 13 times more beetle-to-beetle than tree-to-tree variation. This magnitude was almost double the magnitudes of the ratios among components within the entire plumes. Implications to the behavioral ecology of bark beetle communication, such as potential strategies of cheating and predator avoidance, are discussed.

KEY WORDS aggregation pheromone, *Ips pini*, ipsdienol, monoterpenes, variance components

Chemical communication often involves a complex mixture of signals that include both insect and plant compounds (Landolt and Phillips 1997, Reddy and Guerrero 2004). This is particularly true of bark beetles (Coleoptera: Curculionidae: Scolytinae), which exploit host monoterpenes as precursors and/or synergists of their aggregation pheromones (Wood 1982). These pheromones are multifunctional, serving in mate finding, host procurement, and niche partitioning. In forest ecosystems, however, this signal can be complicated by many factors, such as host and nonhost volatiles, weather, and canopy structure (Raffa 2001, Huber and Borden 2001, Pureswaran et al. 2004). The relative proportions of various monoterpenes and pheromone components can be crucial in how these signals function, because different ratios of the same compounds can elicit a variety of behaviors ranging from increased attraction (Shore and Lindgren 1996, de Groot et al. 1998) to avoidance or masking (Erbilgin and Raffa 2000b, Erbilgin et al. 2003).

For a holistic understanding of semiochemical interactions, the variation in signal production can be as important as the overall mean (Schlyter et al. 2001, Symonds and Elgar 2008, Domingue et al. 2009). For example, variation in the components, timing, and/or strength of signals affects the behavior, evolution, and pest management of bark beetles (Birgersson et al. 1988, Pureswaran et al. 2006). However, patterns and sources of variation are poorly understood and even more poorly quantified (Schlyter and Birgersson 1989, Huber et al. 2004). Variation occurs at a number of levels. For example, chemical components may vary inter-regionally (Birch et al. 1980, Lanier et al. 1980, Miller et al. 1997), within and across tree species (Erbilgin and Raffa 2000a) and among individual beetles (Birgersson et al. 1984, 1988; Zhang et al. 2000; Pureswaran et al. 2008). Despite its importance, few studies have attempted to partition the sources of signal variation quantitatively. Many previous studies examining variation in pheromone response have been performed on beetles from a single host tree or on those exposed to one host monoterpene.

[1] Corresponding author: Canadian Forest Service, Natural Resources Canada, 3333 University Way, Prince George, BC V2N 4Z9 (e-mail: Brian.Aukema@nrcan.g.ca).

[2] University of Northern British Columbia, 3333 University Way, Prince George, BC, Canada V2N 4M3.

[3] Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108.

[4] Department of Biology, Portland State University, PO Box 751, Portland, OR 97207.

[5] Department of Statistics, University of Wisconsin-Madison, 1300 University Ave., Madison, WI 53705.

[6] Department of Entomology, University of Wisconsin-Madison, 345 Russell Laboratories, 1630 Linden Dr., Madison, WI 53706.

Ips pini is a coniferophagous bark beetle distributed across North America. Males construct nuptial chambers in suitable trees and emit aggregation pheromones. The principal pheromone component, ipsdienol, occurs as two stereoisomers [(4*R*)-(−)- and (4*S*)-(+)-2-methyl-6-methylene-2,7-octadien-4-ol]. In some regions, lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one) synergizes attraction to ipsdienol but is not attractive by itself (Teale et al. 1991). Males are joined by two or three females that construct ovipositional galleries. The larvae mine the phloem during development, and pupation occurs under the bark. In the Great Lakes region, the major hosts of *I. pini* are jack, *Pinus banksiana* Lamb, red, *P. resinosa* Aiton, and white, *P. strobus* L. pine (Schenk and Benjamin 1969, Klepzig et al. 1991).

We had two objectives. First, we sought to characterize the volatile plumes of male pine engravers colonizing trees of their three major host species in our region. We examined the proportions of individual monoterpene and pheromone components within these plumes and whether the monoterpene composition within these plumes reflects the constituent monoterpenes in each tree's phloem. Second, we examined how much of the variation in plume components was caused by tree-to-tree (i.e., extrinsic) variation versus beetle-to-beetle (i.e., intrinsic) variation within a given host species.

Materials and Methods

Insects and Host Trees. Three trees each of jack, red, and white pine were collected from a plantation near Mazomanie, WI, on 19 July 2000. Trees were ≈45 yr old and 13–17 cm diameter at breast height (dbh). A 1-m section of each tree was cut from the bole, transported to the laboratory, and maintained in cold storage. On 24 July 2000, 10 evenly spaced holes were drilled just through the outer bark to scratch the phloem layer on each section. A gel capsule containing one male pine engraver was taped over each hole. Random male *I. pini* were sourced from a bulk laboratory colony that was maintained on red and white pines and periodically replenished with wild individuals (Aukema et al. 2004). Only vigorous insects were used. Within each capsule, a 3 by 7-mm fiberglass screen was added to serve as a footing while the beetle began boring. Frass accumulated in the gel capsule as the beetle tunneled. Males that did not enter the log were replaced within 24 h.

Chemical Analyses of Volatiles From Frass. The composition of the volatile plume was determined from the frass of each male beetle. Frass was collected from the gel capsules into small solid phase microextraction (SPME) vials daily for 3 d from the first visible signs of frass as the beetles tunneled. Samples were stored on dry ice immediately after each collection and frozen (−5°C) until processing. The three collections were physically pooled for analysis on a per beetle basis. Headspace analyses of pheromones emanating from the frass were conducted using SPME followed by gas chromatography as previously described (Erbilgin et al. 2003). Frass was placed in 2-ml glass vials with hole-caps fitted with red TFE/Silicone septa (Alltech Associates, Deerfield, IL) and allowed to equilibrate for 1.5 h. The needle of the SPME unit (Supelco, Bellefonte, PA) was inserted through the septum, and the SPME fiber (100 μm polydimethylsiloxane) was exposed for 1 min. The sample was desorbed from the fiber in the injection port of the gas chromatograph (220°C) for 15 s.

All separations were performed on a Shimadzu GLC 14A (Shimadzu Scientific Instruments, Columbia, MD), using a 25-m by 0.25-mm chiral cyclodextrin capillary column (Supelco, Bellefonte, PA). Oven temperature was 60°C for the first 5 min and increased 10°C/min for 5 min to 110°C. The temperature was maintained at 110°C for 17 min and then increased 20°C/min for 5 min to 210°C, which was maintained for 8 min. The carrier gas, helium, was maintained at 30 cm/s. Compounds were identified based on retention times from known samples (Raffa and Steffeck 1988).

Chemical Analysis of Phloem. To determine the constitutive monoterpene profile of each tree, three samples of phloem were removed from each 1-m section of log with a 15-mm cork borer before beetle tunneling. Similar to frass, the phloem disks were stored frozen (−5°C) until processing. The concentration and composition of constituent monoterpenes in the phloem were determined by gas liquid chromatography following the procedure of Powell and Raffa (1999). Each phloem disc was finely chopped with a chisel (≈1-mm^2 pieces) and extracted in 1.5 ml hexane. After 24 h, the extract was removed with a Pasteur pipette and filtered through glass wool. The sample was washed in 400 μl hexane to remove any remaining monoterpenes. The wash solvent was removed with a Pasteur pipette and filtered through glass wool. The volume of extract was brought to 2 ml by addition of hexane. Twenty microliters of 10% paracymene (Aldrich, Milwaukee, WI) was added to each extract as an internal standard.

All separations were performed on a Shimadzu GLC 17A, fitted with an AOC 20i autosampler (Shimadzu Scientific Instruments, Columbia, MD), using a 25-m by 0.25-mm bonded fused silica open tubular polyethylene glycol column (Alltech Associates, Deerfield, IL). Oven temperature was 60°C for the first 10 min and increased 10°C/min for 10 min to 160°C. The carrier gas, helium, was maintained at 30 cm/s. The concentration of each monoterpene was determined by integrating peak areas using Class-VP software (Shimadzu Scientific Instruments). All compounds were quantified by comparing their percentage of the total with the percentage of the internal standard, para-cymene (Raffa and Steffeck 1988). After processing, the phloem was dried and weighed to determine the monoterpene content per gram of phloem.

Statistical Analyses. The concentrations of monoterpenes within the phloem disks were modeled using linear mixed-effects models. Tree species was treated as a fixed effect, and tree was treated as a random effect. A $\log_e(y + 1)$ transformation was used to stabilize the variance. Mean amounts of specific com-

pounds per gram of dry phloem were compared between tree species using protected t-tests using $\alpha = 0.05$ (Carmer and Swanson 1973).

The mean proportions of specific compounds within the volatile plumes were also modeled using linear mixed-effects models. Each host species was analyzed separately. The proportion of each compound within the plume was transformed by $\text{asin}(\sqrt{p})$ and fit to an intercept term. The estimate and SE of the intercept provided inference on the mean proportion and variation in the proportion of the compound being analyzed within each plume. Trees and beetles-within-trees within each species were treated as nested random effects to account for any potential dependence between these randomly selected experimental units (Van Dongen 2000). A variance components analysis estimated the relative sources of the variation arising from tree (i.e., extrinsic) and beetle (i.e., intrinsic) factors by examining the standard errors from the tree and residual (i.e., beetle within tree) nested random effects, represented mathematically

$$\text{asin}(\sqrt{p}) = \beta_0 + \sigma_{\text{tree}} + \sigma_{\text{beetle within tree}}$$

where β_0 estimates the mean proportion of the compound, and the σ_x terms estimate the variation about the estimated proportion attributed to the trees and beetles (Pinheiro and Bates 2002). These estimates were obtained by maximizing the log-likelihood using a Gauss-Hermite approximation (an option in the "lme4" package of R; Ihaka and Gentleman 1996, Bates et al. 2008, R Development Core Team 2009, Bolker et al. 2009). SEs were squared to obtain the variance estimates. Only compounds comprising >10% of the volatile plume were analyzed, because variance estimates of minor components were sometimes difficult to estimate reliably (e.g., confidence intervals varying over several degrees of magnitude). Alternate analytical methods were explored, such as transformations used in compositional data analysis (Aitchison 1986) or logistic regression using compound presence/absence in each signal, but results were invariant between methods so are not reported.

Finally, we examined whether the proportion of specific chemical components of the volatile plume were associated with various constitutive chemicals within the phloem using analysis of covariance (ANCOVA) analyses. The chemical components of interest were the response and covariate(s), respectively, whereas species of host tree was incorporated as a factor. Data were averaged to the level of the individual tree. Examination of residual plots, normal scores plots, and Cook's distances were used as regression diagnostics.

Data from one insect tunneling on jack pine were removed from the analyses because of a recording malfunction on the gas chromatograph. All data analyses were conducted in R using packages "MASS" (Venables and Ripley 2002) and "lme4" (Bates et al. 2008). Density plots used in figures were produced using the densityplot function in the "lattice" package (Sarkar 2007).

Table 1. Mean (± SE) concentrations (μg/g dry phloem) of monoterpenes in phloem tissue of three pine species

Compound	Jack	Red	White	Means comparisons	
				F^a	P
4-Allylanisole	0.0 ± 0.0b	0.0 ± 0.0b	0.3 ± 0.1a	5.24	0.0015
Camphene	0.0 ± 0.0b	0.3 ± 0.2b	1.9 ± 0.3a	11.42	0.0001
3-Carene	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	2.53	0.1052
Myrcene	0.7 ± 0.3a	0.1 ± 0.1b	1.4 ± 0.3c	3.53	0.0485
β-Phellandrene	0.8 ± 0.1c	1.4 ± 0.1b	1.7 ± 0.1a	4.10	0.0323
α-Pinene	13.0 ± 1.5b	14.8 ± 1.6b	45.5 ± 1.3a	4.66	0.0219
β-Pinene	3.6 ± 1.3c	7.3 ± 1.8b	20.3 ± 1.4a	6.60	0.0063
Total	18.3 ± 1.4b	24.4 ± 1.7b	70.6 ± 1.4c	4.96	0.0178

Means across a row (i.e., comparing amounts in different tree species) followed by the same letter are not significantly different (based on 27 samples: 3 phloem samples/tree/species).

[a] df = 2,20.

Results

We detected seven monoterpene compounds within the phloem tissues of jack, red, and white pine (Table 1). Total monoterpene concentrations were highest in white pine, which were approximately four- and threefold times those in jack and red pine, respectively. The predominant monoterpenes were α-pinene and β-pinene, which jointly accounted for 91.7, 91.3, and 92.6% of these monoterpenes in jack, red, and white pine, respectively. All other compounds in the tree species were found in amounts <2 μg/g dry phloem. δ-3-Carene was only detected in red pine, and 4-allylanisole was only detected in white pine.

Table 2. Mean percentage of monoterpene and pheromone components in volatile plumes of frass from male pine engravers from Wisconsin tunneling in three pine species

Volatile proportion of plume	Jack		Red		White	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
All components in plume						
Ipsdienol	50.9	29.1, 72.5	55.5	40.0, 70.4	64.9	51.4, 77.3
All monoterpenes	49.1	27.5, 70.9	44.5	29.6, 60.0	35.1	22.7, 48.6
α-Pinene	31.0	16.0, 48.3	28.6	17.8, 40.7	20.7	13.4, 29.1
β-Pinene	7.5	4.7, 10.8	10.7	7.9, 13.9	9.7	5.4, 15.1
Myrcene	2.0	1.3, 2.8	1.3	1.1, 1.5	1.4	0.7, 2.5
β-Phellandrene	2.9	1.5, 4.7	1.7	1.2, 2.4	1.7	0.09, 2.8
Stereochemistry within ipsdienol component of plume						
(−)-Ipsdienol	30.3	28.5, 32.1	29.8	29.0, 30.7	30.3	29.5, 31.1
(+)-Ipsdienol	69.7	67.9, 71.5	70.2	69.3, 71.0	69.7	68.9, 70.5

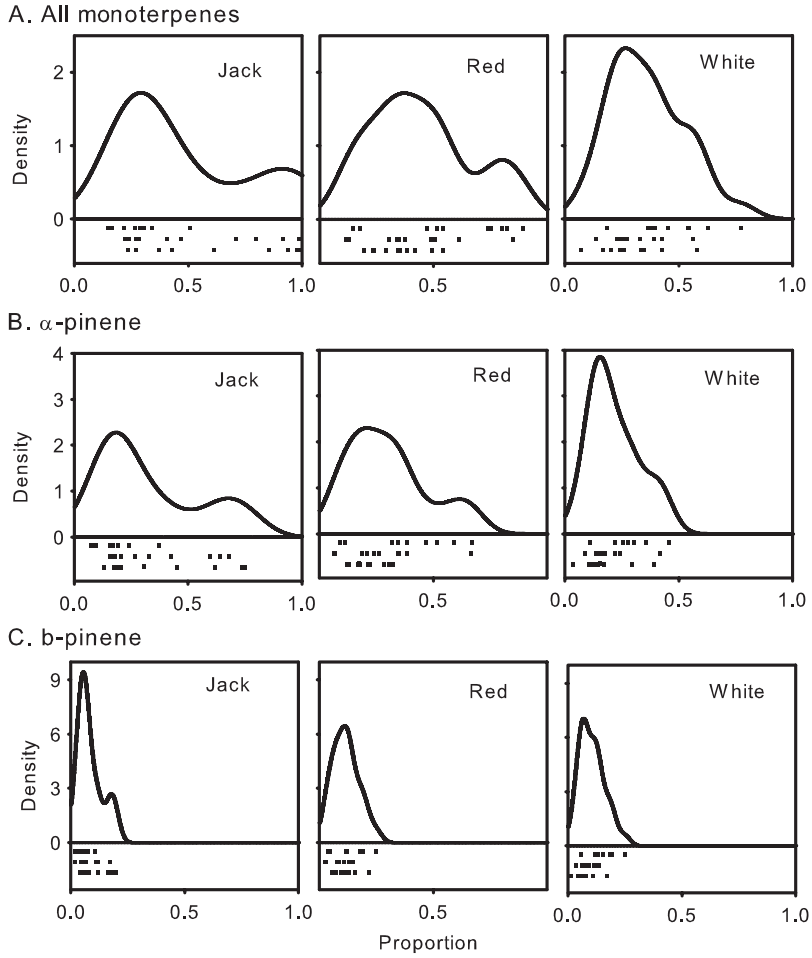

Fig. 1. Probability density functions where the *x*-axis represents the proportions of components of tree volatiles within volatile plumes of male pine engravers tunneling in jack, red, and white pine. The highest peaks in each function indicate the highest relative likelihoods of that proportion(s) of components in the volatile plume (the area under each curve sums to 1). Each of the marks in the three lines below probability density axis ($y = 0$ line) represents an individual beetle. The three rows of marks indicate the proportion of volatile(s) emitted from each of the insects on each of three different trees ($n = 10$ beetles per tree on each of three trees per species). *y*-axis shows proportion of (A) all monoterpenes in plume, (B) α-pinene, and (C) β-pinene. Other components such as myrcene and β-phellandrene make up <5% of the plume and are not shown.

Six volatile components were associated with the tunneling beetles (Table 2). Four of the compounds were from the trees: α-pinene, β-pinene, myrcene, and β-phellandrene (Table 2). The remaining two were the (+) and (–) stereoisomers of the pheromone ipsdienol (Table 2). The strongest ipsdienol signal came from frass of insects tunneling on white pine. Nearly two thirds of this volatile plume consisted of ipsdienol, whereas this pheromone comprised only slightly more than one half of the plume from beetles tunneling in jack or red pine. The largest tree component of the volatile plumes was α-pinene, which comprised approximately one third of the plumes from jack and red pine but only one fifth of the plume from white pine. β-Pinene was the next highest tree component, comprising ≈10% of the red and white pine volatile plumes and ≈8% of the plumes of insects tunneling on jack pine. Other monoterpenes comprised <5% of the volatile plumes. The two components present in trace amounts in the phloem, δ-3-carene and 4-allyanisole, were not detected in volatile plumes. No monoterpenes were present in the plumes that were not detected in phloem.

The overall proportions of components within the volatile plumes were highly variable. For example, the 95% confidence intervals (CIs) of the major components such as α-pinene and ipsdienol could span 25% or more (Table 2). The density plots of the proportions of each component from each beetle's volatile plume on different trees and hosts are shown for monoterpenes in Fig. 1 and ipsdienol in Fig. 2. The smoothed lines in Figs. 1 and 2 are the profiles of the relative proportion of each compound or compound group in the plume over all beetles. The three rows of black dots beneath each figure indicate the proportion of each compound in the plume for each of the 10

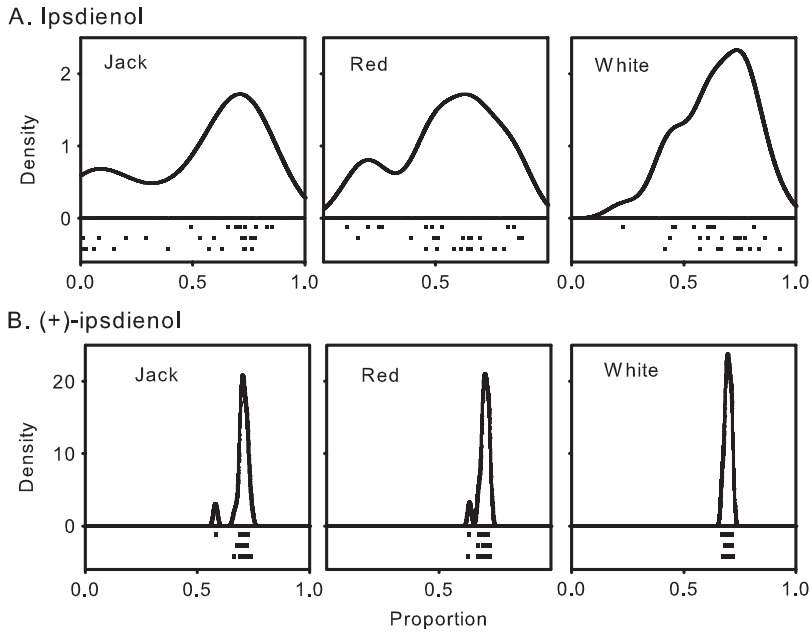

Fig. 2. Probability density functions where the *x*-axis represents the proportions of insect-produced compounds in volatile plumes of male pine engravers tunneling in jack, red, and white pine. The highest peaks in each function indicate the highest relative likelihoods of that proportion(s) of components in the volatile plume (the area under each curve sums to 1). Each of the marks in the three lines below probability density axis ($y = 0$ line) represents an individual beetle. The three rows of marks indicate the proportion of volatile(s) emitted from each of the insects on each of three different trees ($n = 10$ beetles per tree on each of three trees per species). *y*-axis shows proportion of (A) ipsdienol in overall plume and (B) (+)-ipsdienol in the ipsdienol component of the volatile plume.

individual beetle responses, on each of the three trees within the species, to show within-tree and among-tree variation. For example, even though the average signal emanating from male pine engravers tunneling in jack or red pine was ≈50:50 monoterpene:ipsdienol (Table 2), the mode of the signal profile indicates that most volatile plumes consist of less monoterpene (Fig. 1A) than pheromone (Fig. 2A), similar to white pine. On some trees, a few beetles produce very little pheromone, whereas other beetles produce substantially more. In jack pine, the composition of the volatile plumes was between 1.1 and 85.3% ipsdienol; for red pine, between 10.6 and 88.7% pheromone; and for white pine, between 22.8 and 93.2% pheromone. This resulted in distributions that were bimodal or heavily skewed.

The ratio of (+) to (−) ipsdienol within the pheromone component of the plume was highly consistent. Most insects produced a 70 (+) to 30% (−) ratio, although 1 or 2 of the 30 insects in red and jack pine produced a slightly lower ratio (Fig. 2B). 95% CIs were very tight, spanning only 3.5% (Table 2). This consistency is in contrast to the proportions of monoterpene volatiles (Fig. 1) and overall ipsdienol pheromone (Fig. 2A) in the volatile plumes.

The percentage of (+)-ipsdienol in the pheromone component of the volatile plume decreased with increasing concentration of myrcene in the phloem disks ($y = 0.705–0.007x$; $F = 18.34$; df = 1,7; $P = 0.0036$; $R^2_{adj} = 0.684$). Tree species had no effect on this relationship. There was no relationship between the proportion of total ipsdienol, or any monoterpene in the plume, and the concentration of any other monoterpene present in the phloem.

The relative variation caused by intrinsic (i.e., beetle) versus extrinsic (i.e., tree) factors is shown in Fig. 3 for insects tunneling in each of the three host species. Within a species, the beetle-to-beetle variation in plume composition was always greater than tree-to-tree variation, because ratios were always >1. For example, the beetle-to-beetle variation in plume composition of components such as ipsdienol, α-pinene, or β-pinene ranged from 1.3 to 9.3 times greater than the variation attributed to tunneling on different trees (Fig. 3, left three bars each panel).

Despite the consistency of the ratio of (+) to (−) ipsdienol within the pheromone component of the plume (Fig. 2B; Table 2), the little variation that did exist occurred almost entirely at the level of the beetle. The partitioning of variance was similar to that exhibited by components within the overall plumes, but was much more pronounced (Fig. 3, right bar each panel). Within the pheromone component of the plume, there was up to 13 times more variation at the beetle-level than tree-level. For each host species, this was ≈3 times the ratio of beetle:tree variances exhibited by the overall ipsdienol component of the plume (Fig. 3, left bar each panel).

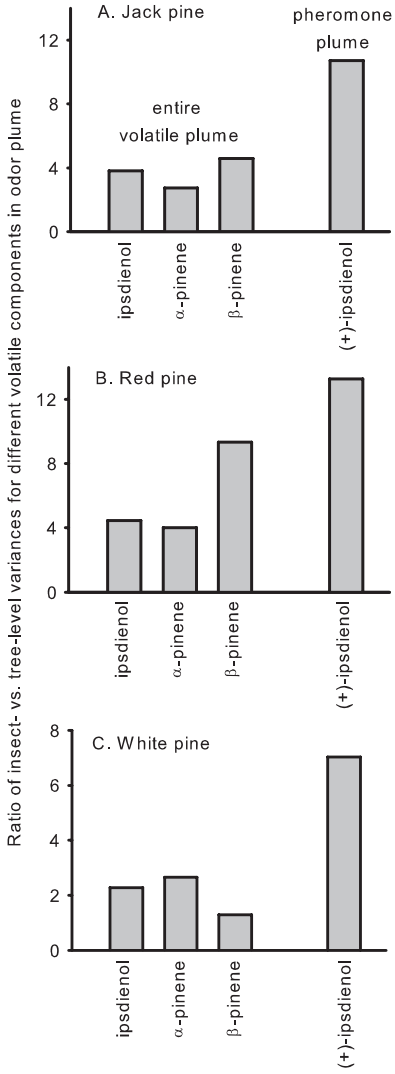

Fig. 3. Ratios of variances attributed to beetle versus tree levels for different proportional components of the volatile plume of male pine engravers tunneling on trees of jack, red, and white pine hosts. For the pheromone component of the volatile plume, the proportion of (+)-ipsdienol [versus (−)-ipsdienol] is shown (right bars). Analysis is based on $n = 10$ insects on each of three trees within each of three host species, with data from one insect on jack pine removed from the analysis.

Discussion

Host species affects the ratio of host compound to insect pheromone within the volatile plumes emitted by tunneling male pine engravers. The highest proportion of pheromone relative to host monoterpene occurred in the signals of insects tunneling in white pine trees. This is consistent with the observation that pine engravers boring into white pines are more attractive than those boring into jack and red pines in the Great Lakes region (Erbilgin and Raffa 2002). Increased attraction may be essential to overcoming resistance in white pine, because the high volume of resin in white pines might otherwise suppress successful attack and colonization. Despite increased attraction by flying beetles to beetles tunneling in white pine, white pine is less commonly attacked than jack or red pines, likely caused in part by the high volume of resin. Moreover, monoterpenes have multiple roles in conifer–bark beetle interactions and may inhibit host entry behavior or continued tunneling at high concentrations (Wallin and Raffa 2000). Thus, the higher concentrations of monoterpenes in white pine (Table 1), and perhaps the presence of 4-allylanisole that can inhibit bark beetle attraction to pheromones (Hayes et al. 1994), might account, in part, for white pine being attacked less commonly in Wisconsin.

The data in Figs. 1 and 2 indicate that insects responding to odor sources are confronted with enormous variation in signal composition, whether conspecific males contributing to both host procurement and intraspecific competition, females seeking mates, or natural enemies exploiting kairomones. The greatest level of variation in volatile plume composition occurs at the level of individual beetles, relative to the tree. Variation in pheromone to monoterpene ratios in the plume was ≈2–9 times greater at the beetle than tree level for most pheromone:monoterpene ratios (Fig. 3). This is likely an underestimate, however, because we used laboratory-reared insects that would presumably exhibit lower variation even though the colony is replenished periodically with wild beetles. Moreover, laboratory analyses of frass do not include host volatiles emanating from wounds created by attacking beetles and resultant induced reactions, volatiles from bark and foliage, and/or climatological effects on the dispersal of these plumes.

Evolution of enantiomeric ratios may be shaped by selective forces such as interspecific competition (Lanier et al. 1980) or predation (Raffa et al. 2007). Our results suggest that any evolution of enantiomeric ratios of pheromone components is not primarily constrained by signal alteration from disparate chemistries of the host trees. Although the correlation between (+)-ipsdienol and myrcene is consistent with previous reports of myrcene being a host-derived precursor (Hughes 1974), we note that the enantiomeric signal within the pheromone component is highly consistent, having a 95% CI of approximately ±1% for each tree species. This consistency exists despite significant variation in constitutive myrcene concentration among tree species (Table 1) and substantial variation in the relative amounts of pheromone and monoterpenes within the volatile plumes from beetle frass (Table 2; Fig. 2B).

Our findings agree with previous reports of intraspecific variation in pheromone emission in bark beetles (Hunt et al. 1986, Birgersson et al. 1988, Pureswaran et al. 2008). This variation can arise from physiological factors such as feeding and fat reserves (Anderbrant et al. 1985, Anderbrant and Schlyter 1989, Vanderwel 1994) and multiple routes of pheromone production, including microbial metabolism (Brand et al. 1975, Leufven and Birgersson 1987), de

novo syntheses (Seybold et al. 1995, Tillman et al. 1998, Nardi et al. 2002, Seybold and Tittiger 2003), or metabolism of host precursors (Hughes 1974, Seybold et al. 2000). Without further experimentation, it is impossible to attribute such variation to specific source(s) in our study, because it is likely caused by both absolute variation in pheromone production from the beetles (Miller et al. 1989, 1996; Pureswaran et al. 2008) and differences in monoterpene emanations from the trees. Although high variation in signal strength can be indicative of the absence of a selective pressure, it could also reflect a strategy of cheating, i.e., a bias by some individuals toward responding to an aggregation rather than initiating new attacks and producing pheromone (Borden et al. 1986, Schlyter and Birgersson 1989). If so, beetles would need to contend with the challenge of balancing absolute signal strength of pheromone components against the relative composition of other compounds in the plume (Table 2). In particular, high ratios of α-pinene to ipsdienol are particularly attractive to the pine engraver's predominant predator in the region, *Thanasimus dubius* F. (Coleoptera: Cleridae) (Erbilgin et al. 2003). This predation can be disproportionately high on late arrivers and could partially offset the host procurement benefits of cheating (Aukema and Raffa 2004).

Recent analyses have documented de novo biosyntheses of ipsdienol that occur after the stimulus of feeding on host compounds (Seybold et al. 1995; Tillman et al. 1998, 2004). Redundancy in pheromone production among bark beetles (Borden et al. 1990, Seybold et al. 2000) minimizes the likelihood that no offspring will be produced (Sih 1980, Wilson and Lessells 1994). Many questions on sources of variation in chemical signaling remain, including whether various host compounds might differentially stimulate different routes of pheromone biosynthesis, the roles of stereoisomers in behavioral responses, and how natural enemies might differentially exploit plant versus insect chemical signals.

Acknowledgments

We thank the Wisconsin Department of Natural Resources for providing the trees. Laboratory assistance by B. Burwitz, L. Leombruni, and J. Ludden was greatly appreciated. This study was supported by NSF DEB-0314215, USDA-NRI WIS04746, McIntire-Stennis, the University of Wisconsin-Madison College of Agricultural and Life Sciences, S.C. Johnson & Son, and an Elsa and Louis Thomsen Wisconsin Distinguished Graduate Fellowship awarded to B.H.A. The authors appreciate the sharp minds and constructive comments of three anonymous reviewers.

References Cited

Aitchison, J. 1986. The statistical analysis of compositional data. Chapman & Hall, London, United Kingdom.

Anderbrant, O., and F. Schlyter. 1989. Causes and effects of individual quality in bark beetles. Holarctic Ecol. 12: 488–493.

Anderbrant, O., F. Schlyter, and G. Birgersson. 1985. Intraspecific competition affecting parents and offspring in the bark beetle *Ips typographus.* Oikos 45: 89–98.

Aukema, B. H., and K. F. Raffa. 2004. Gender- and sequence-dependent predation within group colonizers of defended plants: a constraint on cheating among bark beetles? Oecologia 138: 253–258.

Aukema, B. H., G. R. Richards, S. J. Krauth, and K. F. Raffa. 2004. Species assemblage arriving at and emerging from trees colonized by *Ips pini* in the Great Lakes region: partitioning by time since colonization, season, and host species. Ann. Entomol. Soc. Am. 97: 117–129.

Bates, D., M. Maechler, and B. Dai. 2008. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375–28. (http://lme4.r-forge.r-project.org).

Birch, M. C., D. M. Light, D. L. Wood, L. E. Browne, R. M. Silverstein, B. J. Bergot, G. Ohloff, J. R. West, and J. C. Young. 1980. Pheromonal attraction and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. J. Chem. Ecol. 6: 703–717.

Birgersson, G., F. Schlyter, J. Lofqvist, and G. Bergstrom. 1984. Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* (Coleoptera, Scolytidae) from different attack phases. J. Chem. Ecol. 10: 1029–1055.

Birgersson, G., F. Schlyter, G. Bergstrom, and J. Lofqvist. 1988. Individual variation in aggregation pheromone content of the bark beetle, *Ips typographus.* J. Chem. Ecol. 14: 1737–1761.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M.H.H. Stevens, and J.S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127–135.

Borden, J. H., L. J. Chong, and B. S. Lindgren. 1990. Redundancy in the semiochemical message required to induce attack on lodgepole pines by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera, Scolytidae). Can. Entomol. 122: 769–777.

Borden, J. H., D.W.A. Hunt, D. A. Miller, and K. N. Slessor. 1986. Orientation in forest Coleoptera: an uncertain outcome of responses by individual beetles to variable stimuli, pp. 97–109. *In* T. L. Payne, M. C. Birch, and C.E.J. Kennedy (eds.), Mechanisms in insect olfaction. Oxford University Press, Oxford, United Kingdom.

Brand, J. M., J. W. Bracke, A. J. Markovetz, D. L. Wood, and L. E. Browne. 1975. Production of verbenol pheromone by a bacterium isolated from bark beetles. Nature 254: 136–137.

Carmer, S. G., and M. R. Swanson. 1973. Evaluation of ten pairwise multiple comparison procedures by Monte Carlo methods. J. Am. Stat. Assoc. 68: 66–74.

de Groot, P., G. L. Debarr, and G. Birgersson. 1998. Field bioassays of synthetic pheromones and host monoterpenes for conophthorus coniperda (Coleoptera, Scolytidae). Environ. Entomol. 27: 382–387.

Domingue, M. J., K. F. Haynes, J. L. Todd, and T. C. Baker. 2009. Altered olfactory receptor neuron responsiveness is correlated with a shift in behavioral response in an evolved colony of the cabbage looper moth, *Trichoplusia ni.* J. Chem. Ecol. 35: 405–415.

Erbilgin, N., and K. F. Raffa. 2000a. Effects of host tree species on attractiveness of tunneling pine engravers, *Ips pini*, to conspecifics and insect predators. J. Chem. Ecol. 26: 823–840.

Erbilgin, N., and K. F. Raffa. 2000b. Opposing effects of host monoterpenes on responses by two sympatric species of bark beetles to their aggregation pheromones. J. Chem. Ecol. 26: 2527–2548.

Erbilgin, N., and K. F. Raffa. 2002. Association of declining red pine stands with reduced populations of bark beetle predators, seasonal increases in root colonizing insects, and incidence of root pathogens. Forest Ecol. Manag 164: 221–236.

Erbilgin, N., J. S. Powell, and K. F. Raffa. 2003. Effect of varying monoterpene concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles. Agric. For. Entomol. 5: 269–274.

Hayes, J. L., B. L. Strom, L. M. Roton, and L. L. Ingram. 1994. Repellent properties of the host compound 4-allylanisole to the southern pine beetle. J. Chem. Ecol. 20: 1595–1615.

Huber, D.P.W., and J. H. Borden. 2001. Angiosperm bark volatiles disrupt response of douglas-fir beetle, dendroctonus pseudotsugae, to attractant-baited traps. J. Chem. Ecol. 27: 217–233.

Huber, D.P.W., S. Ralph, and J. Bohlmann. 2004. Genomic hardwiring and phenotypic plasticity of terpenoid-based defenses in conifers. J. Chem. Ecol. 30: 2399–2418.

Hughes, P. R. 1974. Myrcene: a precursor of pheromones in *Ips* beetles. J. Insect Physiol. 20: 1271–1275.

Hunt, D.W.A., J. H. Borden, H. D. Pierce, K. N. Slessor, G.G.S. King, and E. K. Czyzewska. 1986. Sex-specific production of ipsdienol and myrcenol by *Dendroctonus ponderosae* (Coleoptera, Scolytidae) exposed to myrcene vapors. J. Chem. Ecol. 12: 1579–1586.

Ihaka, I., and R. Gentleman. 1996. R: a language for data analysis and graphics. J. Comput. Graph. Stat. 5: 299–314.

Klepzig, K. D., K. F. Raffa, and E. B. Smalley. 1991. Association of insect-fungal complexes with Red Pine Decline in Wisconsin. For. Sci. 41: 1119–1139.

Landolt, P. J., and T. W. Phillips. 1997. Host plant influences on sex pheromone behavior of phytophagous insects. Annu. Rev. Entomol. 42: 371–391.

Lanier, G. N., A. Classon, T. Stewart, J. J. Piston, and R. M. Silverstein. 1980. *Ips pini:* the basis for interpopulational differences in pheromone biology. J. Chem. Ecol. 6: 677–687.

Leufven, A., and G. Birgersson. 1987. Quantitative variation of different monoterpenes around galleries of *Ips typographus* (Coleoptera: Scolytidae) attacking Norway spruce. Can. J. Bot. 65: 1038–1044.

Miller, D. R., J. H. Borden, and K. N. Slessor. 1989. Inter- and intropopulational variation of the pheromone, ipsdienol, produced by male pine engravers, *Ips pini* (Say) (Coleoptera: Scolytidae) J. Chem. Ecol. 15: 233–247.

Miller, D. R., J. H. Borden, and K. N. Slessor. 1996. Enantiospecific pheromone production and response profiles for populations of pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), in British Columbia. J. Chem. Ecol. 22: 2157–2172.

Miller, D. R., K. E. Gibson, K. F. Raffa, S. J. Seybold, S. A. Teale, and D. L. Wood. 1997. Geographic variation in response of pine engraver, *Ips pini*, and associated species to pheromone, lanierone. J. Chem. Ecol. 23: 2013–2031.

Nardi, J. B., A. G. Young, E. Ujhelyi, C. Tittiger, M. J. Lehane, and G. J. Blomquist. 2002. Specialization of midgut cells for synthesis of male isoprenoid pheromone components in two scolytid beetles, *Dendroctonus jeffreyi* and *Ips pini.* Tissue Cell 34: 221–231.

Pinheiro, J. C., and D. M. Bates. 2002. Mixed-effects models in S and S-Plus. Springer, New York.

Powell, J. S., and K. F. Raffa. 1999. Sources of variation in concentration and composition of foliar monoterpenes in tamarack (*Larix laricina*) seedlings: roles of nutrient availability, time of season, and plant architecture. J. Chem. Ecol. 25: 1771–1797.

Pureswaran, D. S., R. Gries, and J. H. Borden. 2004. Antennal responses of four species of tree-killing bark beetles (Cleoptera : Solytidae) to volatiles collected from beetles, and their host and nonhost conifers. Chemoecology 14: 59–66.

Pureswaran, D. S., B. T. Sullivan, and M. P. Ayres. 2006. Fitness consequences of pheromone production and host selection strategies in a tree-killing bark beetle (Coleoptera: Curculionidae: Scolytinae). Oecologia 148: 720–728.

Pureswaran, D. S., B. T. Sullivan, and M. P. Ayres. 2008. High individual variation in pheromone production by tree-killing bark beetles (Coleoptera: Curculionidae: Scolytinae). Naturwissenschaften 95: 33–44.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raffa, K. F. 2001. Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. Chemoecology 11: 49–65.

Raffa, K. F., and R. J. Steffeck. 1988. Computation of response factors for quantitative analysis of monoterpenes by gas liquid chromatography. J. Chem. Ecol. 14: 1385–1390.

Raffa, K. F., K. R. Hobson, S. Lafontaine, and B. H. Aukema. 2007. Can chemical communication be cryptic? Adaptations by herbivores to natural enemies exploiting prey semiochemistry. Oecologia 153: 1009–1019.

Reddy, G.V.P., and A. Guerrero. 2004. Interactions of insect pheromones and plant semiochemicals [review]. Trends Plant Sci. 9: 253–261.

Sarkar, D. 2007. Lattice: lattice graphics. R package version 0.17–2. (http://www.r-project.org).

Schenk, J. A., and D. M. Benjamin. 1969. Notes on the biology of *Ips pini* in Central Wisconsin jack pine forests. Ann. Entomol. Soc. Am. 62: 480–485.

Schlyter, F., and G. Birgersson. 1989. Individual variation of pheromone in bark beetles and moths—a comparison and an evolutionary background. Holarctic Ecol. 12: 457–465.

Schlyter, F., M. Svensson, Q. H. Zhang, M. Knizek, P. Krokene, P. Ivarsson, and G. Birgersson. 2001. A model for peak and width of signaling windows: *Ips duplicatus* and *Chilo partellus* pheromone component proportions—does response have a wider window than production? J. Chem. Ecol. 27: 1481–1511.

Seybold, S. J., and C. Tittiger. 2003. Biochemistry and molecular biology of *de novo* isoprenoid pheromone production in the Scolytidae. Annu. Rev. Entomol. 48: 425–453.

Seybold, S. J., D. R. Quilici, J. A. Tillman, D. Vanderwel, D. L. Wood, and G. J. Blomquist. 1995. De novo biosynthesis of the aggregation pheromone components ipsenol and ipsdienol by the pine bark beetles *Ips paraconfusus* Lanier and *Ips pini* (Say) (Coleoptera: Scolytidae). Proc. Natl. Acad. Sci. U.S.A. 92: 8393–8397.

Seybold, S. J., J. Bohlmann, and K. F. Raffa. 2000. Biosynthesis of coniferophagous bark beetle pheromones and conifer isoprenoids: evolutionary perspective and synthesis [review]. Can. Entomol. 132: 697–753.

Shore, T. L., and B. S. Lindgren. 1996. Effect of ethanol and alpha-pinene on response of ambrosia beetle, trypodendron lineatum, to lineatin-baited funnel and drainpipe traps. J. Chem. Ecol. 22: 2187–2196.

Sih, A. 1980. Optimal behavior: can foragers balance two conflicting demands? Science 210: 1041–1043.

Symonds, M.R.E., and M. A. Elgar. 2008. The evolution of pheromone diversity. Trends Ecol. Evol. 23: 220–228.

Teale, S. A., F. X. Webster, A. J. Zhang, and G. N. Lanier. 1991. Lanierone—a new pheromone component from *Ips pini* (Coleoptera, Scolytidae) in New York. J. Chem. Ecol. 17: 1159–1176.

Tillman, J. A., G. L. Holbrook, P. L. Dallara, C. Schal, D. L. Wood, G. J. Blomquist, and S. J. Seybold. 1998. Endocrine regulation of de novo aggregation pheromone biosynthesis in the pine engraver, *Ips pini* (Say) (coleoptera, scolytidae). Insect Biochem. Molec. 28: 705–715.

Tillman, J. A., F. Lu, L. M. Goddard, Z. R. Donaldson, S. C. Dwinell, C. Tittiger, G. M. Hall, A. J. Storer, G. J. Blomquist, and S. J. Seybold. 2004. Juvenile hormone regulates de novo isoprenoid aggregation pheromone biosynthesis in pine bark beetles, *Ips* spp., through transcriptional control of HMG-CoA reductase. J. Chem. Ecol. 30: 2459–2494.

Van Dongen, S. 2000. Statistical analysis of amounts and ratios in pheromone composition: a mixed model approach. Entomol. Exp. Appl. 94: 269–282.

Vanderwel, D. 1994. Factors affecting pheromone production in beetles. Arch. Insect Biochem. 25: 347–362.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S, 4th ed. Springer, New York.

Wallin, K. F., and K. F. Raffa. 2000. Influences of host chemicals and internal physiology on the multiple steps of postlanding host acceptance behavior of *Ips pini* (Coleoptera: Scolytidae). Environ Entomol. 29: 442–453.

Wilson, K., and C. M. Lessells. 1994. Evolution of clutch size in insects. 1. A review of static optimality models. J. Evol. Biol. 7: 339–363.

Wood, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annu. Rev. Entomol. 27: 411–446.

Zhang, Q. H., G. Birgersson, F. Schlyter, and G. F. Chen. 2000. Pheromone components in the larch bark beetle, *Ips cembrae*, from China: quantitative variation among attack phases and individuals. J. Chem. Ecol. 26: 841–858.

Received 8 October 2009; accepted 20 February 2010.